THE PHYSIOLOGICAL DISPOSITION OF C14-NOREPINEPHRINE IN PATIENTS WITH ATOPIC DERMATITIS AND OTHER DERMATOSES*

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The cause of atopic dermatitis has been variously attributed to allergic mechanisms, psychiatric disorders (1, 2, 13) and an unstable neurovegetative system (2, 13). These, as well as other theories (1, 1A) however, have not afforded satisfactory explanations of this complex disease.

Systemic physiologic and biochemical (1, 4, 5, 6, 7, 11) abnormalities in patients with atopic dermatitis have been observed which suggested to the authors (3) that these patients may have a low level of circulating catecholamines.

Juhlin (8, 9, 10) as well as others (11, 12, 13, 14) have observed abnormalities in the cutaneous vascular physiology of patients with atopic dermatitis which suggest that there may be an alteration of catecholamines in the skin of these patients during a flare of the disease.

These two sets of findings led the authors to hypothesize in a previous paper (3) that under stress, patients with atopic dermatitis bind norepinephrine excessively at the major site of its production (the skin) so that the normally small quantity of norepinephrine which should escape into the circulation does not, and results in a local bound accumulation as well as a circulating deficit of this catecholamine. It was also demonstrated (3), in a small number of patients, that during a flare of the disease, patients with atopic dermatitis have no measurable circulating norepinephrine; and that in remission the plasma levels become normal once more.

The preceding investigation suggested that it would be of interest to determine whether there is any difference in the metabolic fate of intravenously and intracutaneously administered norepinephrine in patients with atopic dermatitis and patients with other dermatoses. Normal values for the excretion of isotopically labelled norepinephrine in normal patients have been established in the past by Goodall, Kirshner and Rosen (27). An attempt to study norepinephrine metabolism in atopic dermatitis was made by measuring the residue of intravenously and intracutaneously injected isotopically labelled dl-norepinephrine acetate in the serum, the amount excreted in the urine as well as the uptake of this material by the skin. A group of patients with acute and chronic atopic dermatitis, as well as patients with other dermatoses and normal skin were studied.§

Subjects: None of the subjects had a past history or presence of hypertension. Except for their cutaneous disorder, all were in good health.

In the first part of this study, four patients with longstanding histories of familial atopy and atopic dermatitis as diagnosed by the usual criteria (1) were studied. The patients were: one Negro age 36 years, 2 Negresses age 15 and 37 years and 1 Caucasian woman age 40 years. The control subjects consisted of 4 Negroes ages 30, 32, 60 and 62 with exfoliative dermatitis due to mycosis fungoides; secondary syphilis with multiple skin lesions (including an acute eczematous reaction due to local overtreatment with heat by the patient) and systemic scleroderma. The last patient was well, but had recovered from a recent upper respiratory infection.

None of the patients tested intravenously were studied by the intracutaneous method. For this part of the study all the subjects were Negro. There were five females, ages 15–34, with mild or moderately severe atopic dermatitis, and one 25-year old man with atopic dermatitis in remission. Six subjects without atopic dermatitis consisted of four men ages 18–40 with mycosis fungoides (exfoliative stage), psoriasis, contact dermatitis and normal skin; and 2 women ages 34 and 50 with exfoliative dermatitis and seborrheic dermatitis respectively.

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METHOD

Intravenous Administration of Isotopically Labelled Norepinephrine: Ten microcuries of beta C 14-dl-norepinephrine acetate (specific activity 20.5 millicuries/mM of norepinephrine–Radiopurity 99.9%, obtained from Nichem, Inc., Bethesda, Maryland) in 500 ml of normal saline were infused intravenously into eight subjects over 20 minutes (0.004 microcuries/kgm/minute, equal to 85 micrograms norepinephrine free base).

The patients voided prior to the onset of the experiment and were kept at bed rest during the entire experiment. Blood pressure readings were recorded every two minutes for the first hour, half-hourly thereafter for the next 6 hours and at least 6 times during the following 18 hours.

Seven to 10 ml of blood for counting was drawn from the arm opposite to which the infusion was given. Samples were taken at 0, 5, 10, 20, 40, 60, 120, 360 and 720 minutes after the end of the infusion. In two patients, samples were taken less frequently but these patients were also tested at 24 hours after infusion. The blood was then refrigerated at 10–14°C. Disposable syringes and needles were used throughout the experiment. Urine was collected at 1, 6, 12 and 24 hours after the infusion was over.

At 24 hours after infusion, an 8 mm punch biopsy specimen of diseased skin was taken from an area of the body (usually the antecubital fossa or very close to it, from the flexural surface of the forearm) opposite to that of the infusion. The tissue was placed in a cotton-stoppered, glass tube, allowed to dry under refrigeration, and was weighed when dry 48 hours later.

Intracutaneous Administration of C-14 Norepinephrine: The skin was anesthetized with 1% lidocaine hydrochloride. A 1 cm round area of diseased skin was marked off with ink (usually the antecubital fossa) and 0.1 ml of diluted C-14 norepinephrine was injected intradermally. One tenth milliliter of this solution was previously determined to contain .007 microcuries of C-14 norepinephrine (or 15,125 disintegrations/min). Exactly 24 hours later, a 6 mm punch biopsy specimen was taken from the center of the previously marked area; and the skin was prepared for counting after being dried and weighed as described above.

Demonstration of the Fate of Norepinephrine by Detecting its Radioactive Carbon Atom in Urine, Serum and Skin

Once the labelled compound had been administered it was necessary to demonstrate its fate by detecting its presence in the serum, its appearance in the urine and its possible retention in the skin. To do this liquid scintillation counting was used (15–22, 26).

Preparation of Samples for Counting

Urine: 0.5 ml of urine was placed in a special counting vial (15) * and 1 drop of 4N HCl was added. To this was added 15 ml of Bray's solution (16). The vial was refrigerated.

Serum: Previous testing showed that there was no more radioactivity in plasma or whole blood than in serum. The blood was allowed to clot and 0.4 ml of clear serum was placed in a counting vial. To this was added 1 ml of hydroxide of Hyamine (21)† and 1 drop of 4N HCl. The mixture was gently agitated until it was clear. When the serum-Hyamine solution was visibly clear, 15 ml of Bray's solution was added, the vial agitated for thorough mixing (1 minute) and the sample placed under refrigeration.

Skin: When the tissue was dry and had been weighed, Hyamine, 1 ml/10 mg (21) of skin (dry weight) was added and the test tube placed in a water bath at 56° C. The specimen dissolved slowly in the Hyamine. When complete dissolution had taken place (8–12 hours) 1 ml of this mixture was drawn off and placed in a vial to which 15 ml of toluene PPO-POPOP mixture‡ was added. The vial was then refrigerated.

Procedure for Counting: The vials containing samples of blanks, urine, serum and skin were placed in a refrigerated Tri-Carb liquid scintillation spectrometer (automatic dual channel model 314 EX-2-Packer Instrument Co.) (15) and permitted to adapt to darkness and cold for 30 minutes. They were then automatically counted at optimum voltage tap for 30–100 minutes each and the count automatically recorded. To minimize counting errors, urine was counted for 30 minutes, serum and skin 100 minutes. Background radiation was determined by counting a blank sample (in its appropriate solvent) with each batch of samples.

Samples with like ratio (1 ± 0.04) were considered to be a similarly quenched group (26) and one sample from each group was internally standardized so that the specific radioactivity of each sample could be calculated. All samples of skin were also standardized.

RESULTS

Atopic Subjects: The four patients with atopic dermatitis excreted 53.5% (mean value) of the administered C-14 norepinephrine load in 24 hours. At 1 hour, 24.9% of the total excreted dose was present in samples from the urine, from 1–6 hours, 39.2%; from 6–12 hours 20.2%; and from 12 hours to 24 hours 15.7% (mean value). Cumulative data may be seen on Graph I.

*Obtained from Packard Instrument Co., La Grange, Illinois.

†Hydroxide of Hyamine 10-x, 1 molar solution in Methanol (trademark of Rohm and Haas, Inc.) is p-(diisobutyl cresoxethoxy ethyl) -dimethyl benzyl-ammonium chloride, a germicidal agent (16, 19, 20)—from Packard Instrument Co., La Grange, Illinois.

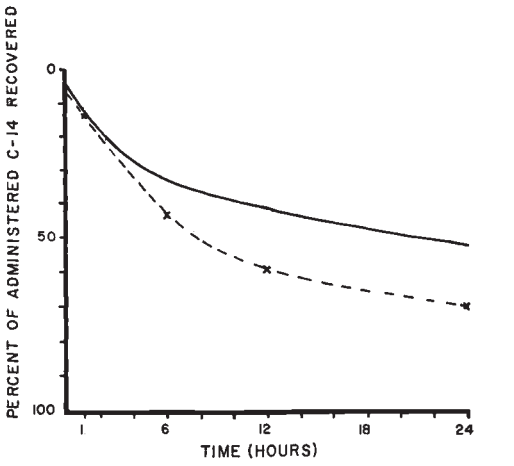
‡ PPO is 2,5-Diphenyloxazolyle. POPOP is (1,4-bis-2-5phenyloxazolyl)-Benzene (18A). The formula for the mixture is PPO, 5.0 g, POPOP 0.3 g, toluene q.s. ad 1 liter.

Non-Atopic Subjects: The four subjects with scleroderma (acrosclerotic type), mycosis fungoides, secondary syphilis and contact dermatitis and the normal, excreted a mean of 70.1% of the administered isotope load (see graph I) in 24 hours. At 1 hour, 21.4% of the total excreted dose was present in samples of urine. In the sample of urine from 1–6 hours after the infusion 42.2% was present, in the 6–12 hour sample 21.2%, and in the 12–24 hour sample 15.2%.

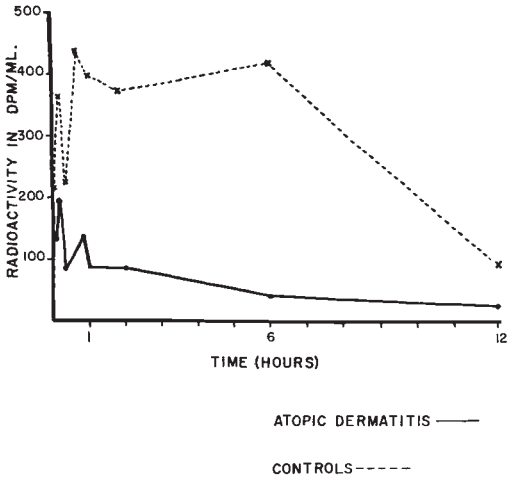
Disappearance of Radioactivity from the Serum

Atopic Subjects: (See Graph II). The radioactivity is expressed as disintegrations per minute (DPM) per milliliter of plasma. The mean radioactivity was found to be 140 DPM/ml at 0 minutes. This rose to a maximum of 195 DPM/ml in 10 minutes and quickly decreased to 85 DPM/ml at 20 minutes, 135 DPM/ml at 40 minutes and 25 DPM/ml at 12 hours. Individual results may be seen in Table III.

Non-Atopic Subjects: (See Table III and Graph II). We do not have as many assayed samples for this group as for the previous group but results are expressed as the mean values for each time sample. At 0 minutes after infusion 216 DPM/ml were found. This reached one peak of 365 DPM/ml at 10 minutes and a second peak of 440 DPM/ml at 40 minutes, then quickly disappeared, so that at 24 hours, no significant radioactivity could be detected.



GRAPH I



GRAPH II

Cutaneous C-14 Norepinephrine Uptake

Non-Atopic Subjects: No significant radioactivity above background could be detected from either the intravenously infused group, or from the intracutaneously treated group except for the patient with psoriasis who retained C-14 equal to two times background.

Atopic Subjects: Radioactivity is expressed in DPM/10 mg. of skin (dry weight) above background (see Tables I and II). (1) Intravenously treated group: Increased radioactivity was detected, correlating roughly, with the degree of cutaneous involvement. This ranged from 34 DPM/10 mg. in a patient with moderate chronic flexural pruritus to 1928 DPM/10 mg. in a patient with longstanding intractable pruritus, generalized flexural lichenification, and eosinophilia of 15%. (2) Intracutaneously treated group: part of the injected dose of isotope was recovered in all but two patients. Again, there was a good correlation between severity and amount of isotope retention. A minimum of 2 DPM's/10 mg. was found in the patient with atopic dermatitis in remission, and a maximum of 878 DPM/10 mg. in a patient with moderately severe atopic dermatitis. One patient with psoriasis retained 52 DPM/10 mg.

Statistics: The results were subjected to a statistical analysis.* The total urinary recovery of C-14 in 24 hours in each patient is expressed as the

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TABLE I

Cutaneous uptake of intravenous norepinephrine

Correction for quenching effect of hyamine-dissolved skin in toluene-PPO-POPOP solution

Diagnosis	Dry Skin Weight (mg)	Observed CPM Minus Background	Increments in CPM's Owing to Addition of Standard*	Per Cent Counting Efficiency	Specific Activity Corrected for Counting Efficiency / Weight × 10 = DPM/10 mgs.
Mycosis fungoides	23	0	9785	46.6%	0
Syphilis II	38	4	8492	40.4%	3
Acrosclerosis	20	0	9030	43.0%	0
Normal	28	0	6480	30.9%	0
Atopic dermatitis, severe	13	948	10449	50.1%	1455.0
Atopic dermatitis, severe	21	1105	5753	27.3%	1928.0
Atopic dermatitis, mild	20	36	11256	53.5%	34.0
Atopic dermatitis, moderate	16	98	8721	41.4%	148.0

* Standard = 21,000 DPM's of C-14 Toluene.

TABLE II

Cutaneous uptake of intracutaneously administered C-14 norepinephrine

Correction for quenching effect of hyamine-dissolved skin in toluene-PPO-POPOP solution

Diagnosis	Dry Skin Weight (mg)	Observed CPM Minus Background	Increments in CPM Owing to Addition of Standard*	Per Cent Counting Efficiency	Specific Activity Corrected for Counting Efficiency / Weight × 10 = DPM/10 mgs.
Atopic moderate	15	78	8032	38.2%	136
Atopic mild	12	50	7113	34.0%	123
Atopic moderate	14	125	8076	38.4%	233
Atopic mild	14	310	9236	43.9%	503
Atopic moderate	18	288	8333	39.6%	878
Atopic in remission	12	1	8897	42.3%	2
Mycosis fungoides	13	0	7650	36.4%	0
Exfoliative dermatitis	11	0	9947	47.4%	0
Psoriasis	17	20	7369	35.1%	52
Contact dermatitis	11	8	9248	44.0%	16
Normal	14	0	8056	33.6%	2
Seborrheic dermatitis	12	4	8532	40.5%	8

* Standard = 21,000 DPM of C-14 Toluene (Packer Instrument Co.).

Percent Recovery of infused C-14. This value is the mean of two separately calculated values: a) the total volume x DPM/ml of a sample from the pooled urine; b) the sums of the volumes x DPM/ml. of samples from 1 hour, 6 hour, 12 hour and 24 hour urines.

The values obtained from the atopic and control groups of patients showed that the atopic patients excreted less C-14 in 24 hours than the non-atopic group. Statistical significance can be inferred, even in small populations, such as this, by an analysis of the t distribution (28), as well as an analysis of variance (29). The differences between the two groups of patients studied was found to be significant at the 1 percent level for both the t distribution, as well as an analysis of variance.

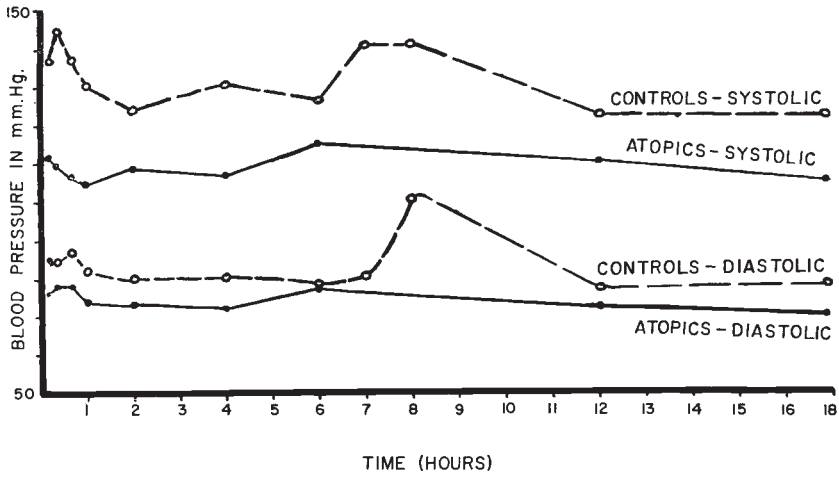
Clinical Effects (See Graph III)

Repeated blood pressure readings during and after the infusion showed that in the non-atopic patients, the mean blood pressure readings initially rose from a basal reading of 128/80 mmHg in twenty minutes to 145/85 mmHg. This returned to pre-infusion levels in 1–2 hours. At 6 hours after infusion there was a second rise to

TABLE III

Radioactivity in serum after intravenous infusion of C-14 norepinephrine (expressed in DPM/cc)

Time after End of Infusion	Atopic Dermatitis	Atopic Dermatitis	Atopic Dermatitis	Atopic Dermatitis	Secondary Syphilis	Mycosis Fungoides	Acroscler-osis	Normal
0 Minutes			142	123	216			
5 Minutes			146	115				361
10 Minutes		323	146	115				
20 Minutes	69	161	115	85			90	353
40 Minutes	23	355	85	81			750	125
60 Minutes	42	139	85	85	354	180	850	208
2 Hours	55	152	65	65			550	196
6 Hours	13	65	38	46	455	599	0	188
12 Hours	4	55	27	27	202	30	0	39
24 Hours					0	24	0	0



GRAPH III

153/88 mmHg which slowly returned to local levels by 12 hours.

In the atopic group, all patients had low-normal or low initial readings of 107/74 mmHg (mean value). After infusion, there appeared to be a small rise to 110/79 mmHg which remained at that level until 12 hours when it returned to pre-testing levels.

DISCUSSION

Problems Involved in Preparation of Specimens: The preparation of organic matter for determination of radioactivity presents two large problems. A solvent must be found which will permit dissolution of the radioactive sample and scintillator. At the same time it must give minimal quenching. Since urine is aqueous, dried skin largely protein in nature and serum an aqueous-protein complex, it was obvious that the same solvent could not be used for all the samples. Therefore, we searched for a solvent of aqueous systems (urine) as well as one for proteins (skin) and combined elements of both to measure radioactivity in serum.

Bray (16) has described a mixture containing naphthalene, PPO, POPOP, methanol, ethylene glycol and p-dioxane, which apparently has excellent aqueous solvent properties. We applied this system to the study of C-14 norepinephrine and its catabolites excreted in urine.

Hyamine, developed by Passmann, Radin and Cooper (20), soluble itself in toluene, proved itself to be an excellent sclvent of proteins (19) and we used it as our solvent for skin. We com-

bined the use of Hyamine with Bray's solution in studying serum. This resulted in counting efficiencies of about 25% for serum, 35% for urine and 40% for skin. Besides the problem of quenching, there is of course a loss of countable disintegrations per minute inherent in the counting system itself. The efficiency of the spectrometer used in this experiment was found to be 58% ± 2%. The total DPM of any given sample, therefore, was calculated from the counts per minute obtained (mean of 100 minutes) taking into account quenching and operating efficiency of the spectrometer and subtracting the background radiation as measured by counting blank samples of serum, urine and skin.

Interpretation of Results: The total 24-hour urinary recovery of the administered C-14 norepinephrine in the atopic group was found to be 16.6%, less than non-atopic group (See Graph I) although the same dose was administered to both groups under similar conditions. There was a concomitant finding of increased radioactivity in the skin of the atopic patients as compared with controls. There was also a rough correlation between the degree of dermatitis and the amount of norepinephrine retention. It seems reasonable to conclude that the diminished excretion was due to a sequestration of norepinephrine in the skin of atopic dermatitis.

There was no significant retention of radioactivity in the skin of patients with a variety of dermatoses other than atopic dermatitis except in a case of psoriasis where the radioactivity was found to be slightly increased. The rates of disappearance of radioactivity from the serum were less conducive to unequivical interpretation.

It is possible that the norepinephrine is bound immediately after the intravenous infusion into atopic skin, then very slowly released. This could account for the two types of curves of radioactivity we discovered in the serum of the two groups of patients. In the atopic group, the serum level of radioactivity at first was found to be very low, then a small rise was detected (195 DPM/ml). This was followed by a second peak to 135 DPM/ml followed by a slow decrease. There was still detectable radioactivity at 12 hours after the infusion. This last described curve of radioactivity probably represents catabolic products of norepinephrine metabolism rather than norepinephrine itself (35).

In the non-atopic group detectable radioactivity was highest within the first 60 minutes after the intravenous injection. At 12 hours none could be detected. This probably represents the normal sequence of events: infusion → presence in serum → uptake in tissues (low serum C-14) → catabolism (high serum C-14) → excretion (appearance in urine). The difference between the two groups is probably one of delay of excretion after prolonged retention in atopic dermatitis skin. This supposition is further strengthened by the clinical findings of an immediate rise in blood pressure followed by a delayed rise in the non-atopic group, compared to a minimal steadily maintained 12 hour rise in the atopic group. The $\frac{\text{fractionated percents}}{\%\text{ total recovery}}$ of administered C-14 excreted during the first hours, during the next 5 hours, the following 6 hours and the last 12 hours was not found to be different from one group to the next.

Theoretical Implication: Patients with acute atopic dermatitis were found to sequester norepinephrine in the skin at 24 hours after intravenous infusion, 2–50 times more than patients with a variety of other eczematous and non-eczematous dermatoses. The degree of cutaneous C-14 retention also coincided with the clinical severity of the dermatitis. The sequestration of intravenously administered C-14 norepinephrine coincided with a decreased level in the serum and diminished excretion in the urine of these patients. Goodall, Kirshner and Rosen (27, 35) have shown that the radioactive products of infused C-14 norepinephrine in the normal human is excreted at the rate of 67 ± 4% in 24 hours. They separated the various catabolic fractions in the urine into 10 fractions. Our normal subjects showed a total excretion of radioactivity very similar to those described by Goodall and his collaborators (35), but the atopic group excreted a mean of 16.6% less. This was paralleled by an increased retention of the catecholamine in the skin of these patients.

Intracutaneous administration of C-14 norepinephrine again showed a marked retention in the skin of patients with acute atopic dermatitis as compared to non-atopic dermatitic skin. These findings would seem to strengthen our hypothesis of a cutaneous binding affinity for catecholamines in acute atopic dermatitis.

The presence of norepinephrine in human and animal skin has been demonstrated by Moller

and Hakanson (25, 36). We can postulate on the basis of past observations as well as these studies on the type of bond which holds the norepinephrine. Biochemical and pharmacological evidence has been accumulating that indicates the in vivo presence of several forms of bound norepinephrine (23). Of the total bound norepinephrine (or norepinephrine "store") a portion may be released by tyramine and other sympathomimetic amines. In addition to this easily released norepinephrine, there is a tightly bound store which can be released by reserpine or guanethidine. A third part of the store is available for release by nerve stimulation and cannot be depleted by tyramine. Besides these pharmacologically designated forms of bound norepinephrine, Burn has postulated and shown that acetylcholine may also release norepinephrine (24). Topical application of cortico-steroid creams to the skin have been shown to cause vasoconstriction (30, 34). It is possible, as well, that cortico-steroids may be another source of norepinephrine release from a bound state. It would seem reasonable to conclude from our findings here, the presence of an excess store of tightly bound form of norepinephrine in skin of patients with atopic dermatitis. It should however be noted that histochemical evidence for the presence of catecholamines in human skin is equivocal (31, 32, 33).

It appears evident from the results of this study that further work in this fertile field of cutaneous catecholamine metabolism may yield valuable information both for the basic scientist and the clinician.

SUMMARY

The metabolism of intravenously infused and intracutaneously injected C-14 norepinephrine acetate was studied in 10 patients with atopic dermatitis and in 10 patients with other eczematous dermatoses, scleroderma, psoriasis, and normal skin.

The mean urinary excretion expressed as percent of the administered load of norepinephrine and its catabolites was 16.6% less in 4 patients with atopic dermatitis than in 4 control subjects (53.5% vs. 70.1%). This difference was statistically significant at the 1% level.

The administered isotope tends to disappear from the plasma in a different fashion in the atopic group than in the control group. C-14 norepinephrine, 24 hours after intravenous administration, was found to be 2 to 50 times higher in the skin of the atopic group as compared to the control group.

Intracutaneously administered norepinephrine was also found 24 hours later in the skin of patients with atopic dermatitis to be 3 to 5 times higher than in a variety of other dermatoses and normal skin.

The technical difficulties of assaying administered isotopically labeled substances in skin, serum and urine are discussed. The results of this experiment indicate that in acute atopic dermatitis norepinephrine (in excess of normal) appears to be found tightly in the skin.

REFERENCES

1. Baer, R. L.: Atopic dermatitis. Med. Clin. N. Amer., **43:** 3, 770, 1959.
1A. Hashem, N., Hirschorn, K., Sedlis, E. and Holt, L. E., Jr.: Infantile Eczema, evidence of autoimmunity to human skin. Lancet, **2:** 269, 1963.
2. Kalz, F., Wittkower, E. D., Vavruska, G. W., Telner, P. and Ferguson, F.: Studies on vascular skin responses in atopic dermatitis. J. Invest. Derm., **29:** 67, 1957.
3. Solomon, L. M. and Wentzel, H. E.: Plasma catecholamines in atopic dermatitis. J. Invest.Derm. **4:** 401, 1963.
4. Sternberg, Th.H. and Zimmerman, M. C.: Stress studies in eczema-asthma-hay fever diathesis. Arch. Derm. (Chicago), **65:** 392, 1952.
5. Eyster, W. H., Jr., Roth, G. M. and Kierland, R. R.: Studies on the peripheral vascular physiology of patients with atopic dermatitis. J. Invest. Derm., **24:** 19, 1955.
6. Weber, R. Y., Roth, G. M. and Kierland, R. R.: Further contributions to the vascular physiology in atopic dermatitis. J. Invest. Derm., **24:** 19, 1955.
7. MacKenna, R. M. B. and Lehman, H.: The glucose-tolerance test in skin disease. Lancet, **1:** 1393, 1963.
8. Juhlin, L.: Skin reactions to iontophoretically introduced epinephrine and norepinephrine in atopic dermatitis. J. Invest. Derm., **37:** 201, 1961.
9. Idem: The fate of iontophoretically introduced epinephrine and norepinephrine in patients with atopic dermatitis and in normal skin. J. Invest. Derm., **37:** 257, 1961.
10. Idem: Vascular reactions in atopic dermatitis. Acta Dermatovener. (Stockholm), **42:** 218, 1962.
11. Lobitz, W. C., Jr. and Campbell, C. J.: Physiologic studies in atopic dermatitis: disseminated neurodermatitis. I. The local cutaneous response to intradermal injected acetylcholine and epinephrine. Arch. Derm. (Chicago), **65:** 575, 1953.
12. Stokes, J. H., Ingraham, N. and Beerman, H.: The psychoneurogenous component of cutaneous reaction mechanisms. Amer. J. Med. Sci., **198:** 577, 1939.

13. STORCK, H. AND HEIM, E.: Circulatory and neurovegetative changes in atopic and eczematous patients. Schweiz. Med. Wschr., **92:** 162, 1962.
14. VIGLIOGLIA, P. A.: Guanethidine as an antipruritic. Arch. Derm. (Chicago), **85:** 472, 1962.
15. BELL, C. G. AND HAYES, F. N.: Liquid Scintillation Counting. London, Pergamon Press, 1958.
16. BRAY, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem., **1:** 4, 279, 1960.
17. RAPKIN, E.: The determination of radioactivity in aqueous solutions. Packard Tech. Bull., **6:** 1, March, 1963.
18. HAYES, F. N.: Solutes and solvents for liquid scintillation counting. Packard Tech. Bull., 1 (Revised): 1, April, 1963.
18A. IDEM: 1, November, 1960.
19. RAPKIN, E.: Hydroxide of hyamine 10-X. Packard Tech. Bull., **3:** 1, 1961.
20. PASSMANN, J. M., RADIN, N. S. AND COOPER, J. A. D.: Liquid scintillation technique for measuring carbon-14-dioxide activity. Anal. Chem., **28:** 484, 1956.
21. VAUGHAN, M., STEINBERG, D. AND LOGAN, J.: Liquid scintillation counting of C-14 and H-3 labeled amino acids and proteins. Science, **126:** 446, 1947.
22. FARMER, E. C. AND BERNSTEIN, I. A.: Determination of specific activities of C-14 labeled organic compounds with a water-soluble liquid scintillator. Science, **115:** 460, 1952.
23. KOPIN, I. J. AND AXELROD, J.: The role of monoamine oxidase in the release and metabolism of norepinephrine. Ann. N. Y. Acad. Sci., **107:** Art. 3, 848, 1963.
24. BURN, J. H.: The release of norepinephrine from the sympathetic postganglionic fiber. Bull. Hopkins Hosp. **112:** 167, 1963.
25. MOLLER, H.: Catecholamines of the skin in man. Acta Dermatovener. (Stockholm), **42:** 386, 1962.
26. CHASE, G. D.: Principles of radioisotope methodology, PP. 147. Minneapolis, Burgess Publishing Co., 1959.
27. GOODALL, MCC., KIRSHNER, N. AND ROSEN, L.: Metabolism of noradrenaline in the human. J. Clin. Invest., **38:** 707, 1959.
28. WILKS, S. S.: Elementary Statistical Analysis, PP. 212. Princeton, New Jersey, Princeton Univ. Press, 1949.
29. DIXON, W. S. AND MASSEY, F. J., JR.: Introduction to Statistical Analysis. PP. 102 and ff., New York, McGraw-Hill Book Co., Inc. 1957.
30. MCKENZIE, A. W. AND STOUGHTON, R. B.: Method for comparing percutaneous absorption of steroids. Arch. Derm. (Chicago), **86:** 608, 1962.
31. ADAMS-RAY, J. AND NORDENSTAM, H.: Un Systeme de Cellules Chromaffines dans la Peau Humaine. Lyon Chir., **52:** 125, 1956.
32. MONTAGNA, W.: The Structure and Function of the Skin. New York, Academic Press, Inc., 1956.
33. MERCANTI, E. S.: Failure to show the presence of a chromaffin system of cells in the human skin. J. Invest. Derm., **34:** 317, 1962.
34. ASHTON, N. AND COOK, C.: In vivo observations of the effects of cortisone upon the blood vessels in rabbit ear chambers. Brit. J. Exp. Path., **33:** 445, 1952.
35. GOODALL, MCC. AND ROSEN, L.: Urinary excretion of noradrenaline and its metabolites at ten-minute intervals after intravenous injection of dl-noradrenaline-2-C^{14}. J. Clin. Invest., **42:** 1578, 1963.
36. HAKANSON, R. AND MOLLER, H.: Uptake of catecholamines in the skin. Acta Dermatovener. (Stockholm), **43:** 343, 1963.